

## Diastatic activity in some unifloral honeys

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**Summary** — Determinations of diastatic activity in 12 groups of unifloral honey were made to study variability according to the botanical origin of the honey. *Robinia*, *Citrus*, *Erica*, *Taraxacum* and *Arbutus* honeys were found to have a very low enzyme content. On the contrary *Hedysarum*, *Castanea*, Honeydew, *Eucalyptus* and *Thymus* honeys showed high diastase activity. The relationship between the absorbance at 5 min and the diastatic index was quantified.

**honey / enzyme activity / amylase / absorbance**

### INTRODUCTION

The presence of enzymes in honey has been known for many years. One of the better known enzymes is diastase or amylase. The origin of this enzyme in honey has been attributed to the salivary secretions of bees (Gothe, 1914), or to its presence in pollen (Vansell and Freeborn, 1929; Lothrop and Paine, 1931), or nectar (Fiehe, 1932; Gorbach, 1942). Today, the most widely accepted theory attributes the origin of diastase in honey to salivary secretions of bees. This conclusion is based on the presence of diastase in honey produced by sugar-fed bees, and on the similarity between honey diastase and bee diastase (Ammon, 1949; Rinaudo *et al*, 1973; Stadelmeier and Bergner, 1986). However, this does not explain why honeys of diverse botanical origin show a different diastatic activity, a fact which has been known for many years (Lothrop and Paine,

1931). Various explanations for the low enzymatic activity of certain honeys have been proposed, such as a poor processing of nectar by the bees during an abundant nectar flow (Sipos, 1964), or seasonal activity of the pharyngeal glands (Halberstadt, 1980; Fluri *et al*, 1982). Even international standards include honeys with "a low natural content of enzymes" for which different limits are accepted (CAC, 1969).

Another extensively studied aspect of diastase activity in honey is its susceptibility to temperature and age of the honey. Although diastase sensitivity to heat and storage is not very high compared to saccharase (White *et al*, 1964), the measurement of diastatic activity is used to evaluate the freshness of honey. The qualitative parameters of the European standards (CAC, 1969) prescribe a diastatic index of no less than 8 on the Gothe scale and no less than 3 for honeys with a low natural enzyme content.

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The present study describes variability of diastase activity in relation to the botanical origin of the honey. In addition, to contribute to the simplification of the lengthy chemical analysis, the relationship between the diastatic index of a honey and the absorbance measured at the start of the analysis (5 min) was quantified.

## MATERIALS AND METHODS

During an extensive study of characteristics of Italian honeys (Accorti *et al*, 1986), 625 honeys of diverse botanical origins, produced in different years and in various Italian regions were analyzed for diastase. From these, 343 were chosen which, without doubt, could be classified as unifloral honeys. The choice was based on organoleptic characteristics (taste, smell, colour, physical state), physico-chemical properties (electrical conductivity, specific rotatory power, total acidity, pH, glucide spectrum) and microscopic characteristics (qualitative and quantitative melissopalynological analysis) according to the limits described by Accorti *et al* (1986).

All the samples had been refrigerated ( $-20^{\circ}\text{C}$ ) and were analyzed within 12 months of extraction. Their freshness at the time of analysis was verified through the determination of HMF ( $< 10\text{ mg/kg}$ ).

The selected samples were distributed as follows: 92 *Castanea* unifloral honeys, 76 *Robinia*, 29 *Hedysarum*, 23 *Eucalyptus*, 22 *Arbutus*, 18 *Citrus*, 15 *Helianthus*, 11 *Thymus*, 9 *Erica*, 9 *Rhododendron*, 9 *Taraxacum* and 30 Honeydew from spruce fir. The diastatic activity was determined for the entire assay according to the method of Schade *et al* (1958), modified by White and Pairent (1959) and by Hadorn (1961), accepted as the official method of analysis (CAC, 1969). Merck 1252 soluble starch was used after the blue value was verified. The results are expressed in Gothe scale units. The data collected were introduced into a program of simple descriptive processing.

## RESULTS

The total variability of the diastatic index encountered in the assay (625 honeys) covered a vast range, from a minimum of zero to a maximum of 43.5 (average  $18.3 \pm 9.1$ ). Among the types analyzed, *Erica*, *Robinia*, *Taraxacum* and *Citrus* have a low diastatic index, with an average of 8–10 (fig 1 and table I). Particularly low values (average  $5.2 \pm 3.0$ ) were encountered for the diastatic activity of *Arbutus* honeys, 2 samples of which gave a value of zero. These

**Table I.** Diastatic index values (expressed in Gothe scale units) for the principal Italian unifloral honeys.

Botanical origin	No samples	Mean	St dev	Min	Max
<i>Arbutus</i>	22	5.2	3.0	0	9.2
<i>Erica</i>	9	7.8	4.9	3.7	18.2
<i>Robinia</i>	76	8.4	2.9	3.1	15.0
<i>Taraxacum</i>	9	9.2	4.9	3.4	17.4
<i>Citrus</i>	18	9.8	3.0	3.4	16.3
<i>Rhododendron</i>	9	13.7	2.4	9.1	16.7
<i>Helianthus</i>	15	16.3	3.2	8.7	20.3
<i>Hedysarum</i>	29	19.8	6.0	12.5	33.3
Honeydew	30	22.9	6.9	10.9	34.1
<i>Castanea</i>	92	24.1	5.2	10.6	42.9
<i>Eucalyptus</i>	23	25.5	4.2	16.2	34.9
<i>Thymus</i>	11	33.1	4.7	24.3	39.0

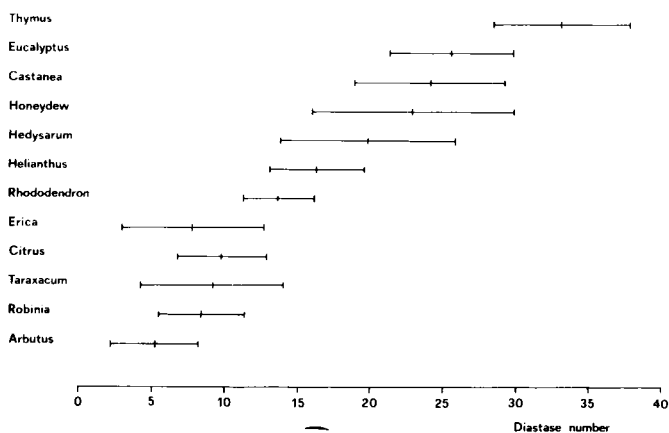


Fig 1. Diastatic activity (average  $\pm$  standard deviation) in the 12 groups of unifloral honeys analyzed.

honeys do not, therefore, come within the limit of 3 that the law prescribes for honeys with a low enzyme content, unlike the previous types for which the minimum values were always greater than 3.

It must be pointed out that the samples were fresh and that none of them had been heated, as confirmed by the HMF values that were all extremely low. So, the obtained values can be considered as characteristic of the examined honeys and not dependent on external factors.

In the other unifloral types, *Rhododendron* and *Helianthus* showed moderate values (with an average of 13.7 and 16.3 respectively); *Hedysarum*, *Castanea*, *Honeydew* and *Eucalyptus* were characterized by a high diastatic index, with average values of 20–25. Diastatic activity was exceptionally high in *Thymus* honeys (average =  $33.1 \pm 4.7$ ).

A comparison of these results with those reported in the literature shows that while numerous data are available for some honeys, they are very sparse or totally lacking for others. The general average diastatic index found for the entire as-

say (18.3) coincides fairly well with that reported by White *et al* (1962) for an assay of some 500 American honeys (20.8), although their overall range covered much higher values (2.1–61.2).

For the unifloral types the most abundant data are found for *Robinia* and *Citrus* honeys. For *Robinia*, all authors give rather low values, similar to those found in this research (White *et al*, 1962; Fini and Sabatini, 1971; Marletto *et al*, 1977; Patetta *et al*, 1977; Ivanov, 1978). Low values have also been reported for *Citrus* honeys (White *et al*, 1962; Skender, 1972; Fini and Sabatini, 1974) and are supported by our results. Only those obtained by Serra Bonvehi *et al* (1986) indicate a higher average value, of  $21.8 \pm 4.6$ .

The scanty data available for the other unifloral types are often contradictory: for instance, the reported average diastatic index values for *Eucalyptus* honeys are 18 (Serra Bonvehi and Cañas Lloria, 1988), 21.9 (White *et al*, 1962), 29.6 (Langridge, 1966) and 43 (Fini and Sabatini, 1974), whereas for Honeydew honeys, White *et al* (1962) give 6.7 to 48.4, and Serra Bonvehi *et al* (1986) give an average of  $50.2 \pm 10.7$ .

The different values obtained by various authors may be attributed, at least in part, to the procedure adopted for the analysis, ie the type of starch used (Piazza and Accorti, 1981). In certain cases, however, we also encountered a considerable difference between minimum and maximum values within the same unifloral group, particularly for *Castanea* and Honeydew honeys (table I).

Values that grossly contradict ours and that are difficult to explain have been reported by Thrasyvoulou (1986), who in 125 samples of Greek honey encountered the lowest diastatic values for *Thymus* honeys, both for their overall range (4.5–35.2) and for their average (15.6). An explanation for such a great difference could be that the analysis may have concerned different species of *Thymus*. Also seasonal or bee-dependent factors might have played a role.

#### *Relationship between initial diastatic index and absorbance*

It takes a relatively long time to carry out analyses to determine diastatic activity, particularly for honeys with low values.

It is a well-known fact that the 5-min value gives an approximation of the end point (AOAC, 1980; Méthodes Officielles d'Analyse du Miel, 1977), and it was suggested that a definite relationship can be established between the diastatic index of a sample and the absorbance read at 5 min (Mohamedally, 1979, quoted by Wix, 1980). This could be a useful element since it would enable us to reduce the number of readings and the time necessary for analysis.

Consequently, since we had 281 paired observations of 5-min absorbance and diastase values we attempted to quantify this relationship. The results are expressed in the following linear equation (figure 2) :

$$X_2 = 70.1804 - 95.4183 X_1$$

where:  $X_2$  = diastatic index;  $X_1$  = absorbance at 5 min.

The correlation between  $X_1$  and  $X_2$  is highly significant ( $r = -0.94949$ ,  $P < 0.001$ ), and also the angular coefficient is significantly different from 0 ( $P < 0.001$ ). However, a more detailed analysis of the correlation within each group of unifloral honeys

**Table II.** Values of the regression between diastatic index and absorbance at 5 minutes for the different unifloral groups and their comparison with the values obtained for the total assay. \*\* = significant for  $P < 0.001$ ; ns = not significant).

Botanical origin	No	a	b	r	sign r	$t_b$	sign b
<i>Erica</i>	9	70.6480	-96.0900	-0.9591	**	0.4654	ns
<i>Hedysarum</i>	20	71.3045	-96.6951	-0.9719	**	0.4654	ns
<i>Robinia</i>	52	67.0579	-92.7342	-0.9352	**	-1.0252	ns
Honeydew	49	71.0069	-99.0081	-0.9052	**	1.2192	ns
<i>Thymus</i>	10	70.6941	-88.4858	-0.8927	**	-1.6488	ns
<i>Helianthus</i>	32	68.1127	-89.2783	-0.9344	**	-2.1569	ns
<i>Castanea</i>	55	66.1425	-87.1780	-0.8597	**	-2.7462	**
<i>Eucalyptus</i>	16	76.8465	-108.1093	-0.9491	**	3.7746	**
<i>Citrus</i>	22	39.6945	-46.7763	-0.6414	**	-12.9554	**
Total	281	70.1804	-95.4183	0.9495			

shows that the lines representing *Castanea*, *Eucalyptus*, and particularly *Citrus* honeys (table II) are considerably detached from the general line. *Arbutus*, *Rhododendron* and *Taraxacum* honeys were represented by an insufficient number of data for any conclusion.

This induces us to consider that the phenomenon might be explained more satisfactorily by a non-linear model. However, since our objective is basically practical, we consider the linear model sufficient for forecasting the time intervals of the readings after the first at 5 min.

## CONCLUSIONS

Our data show that the diastatic activity of the honey is extremely variable among groups of unifloral honey. This variability is probably due to a series of uncontrollable intrinsic and extrinsic factors, and can be found also to a remarkably lesser degree, within the groups of unifloral honeys. However, despite this, the honeys with a low enzyme content are clearly distinguished from those with a moderate or high content.

An important remark must be made concerning the validity of the limit of 8 units (3

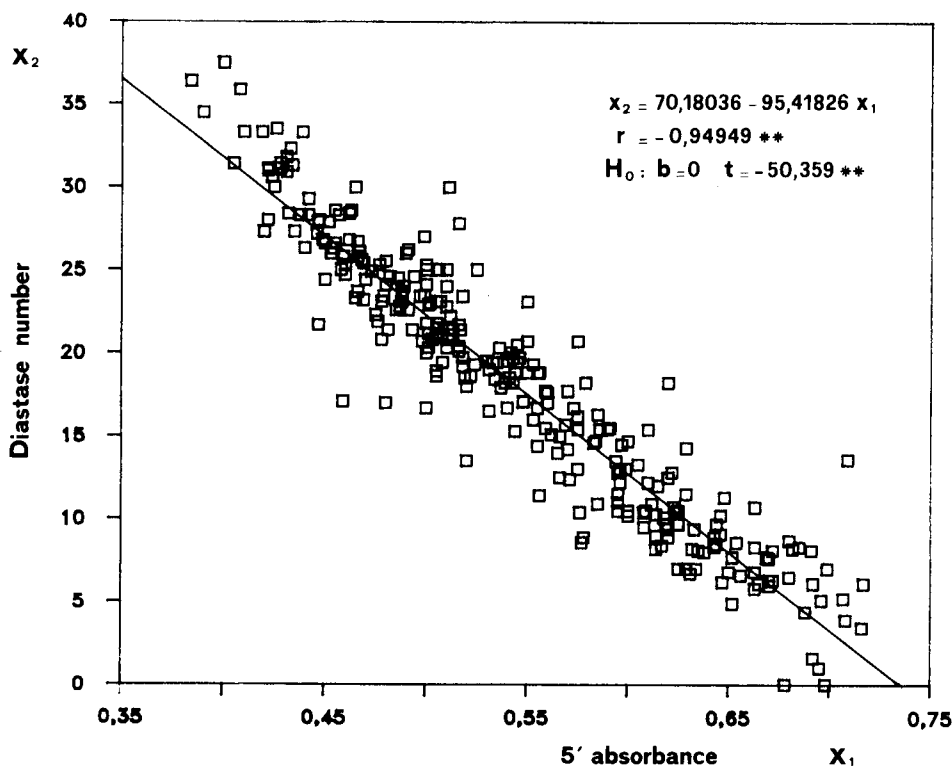


Fig 2. Relationship between diastatic index and absorbance at the first reading (5 min) on 281 couples of points.  $X_1$  = absorbance at 5 min;  $X_2$  = diastatic index.

for honeys with a low natural enzyme content) prescribed for diastase activity by the international standards. With the large variation in values found in this study, the determination of diastatic activity cannot be considered a valid criterion to measure the freshness of honey. Unlike the HMF content which is theoretically zero in a fresh honey, it is impossible to know the original diastase content. Consequently, the same value can have a different meaning according to whether the original content was high or low. Unless other information is provided, it is therefore impossible to give a correct interpretation of the measured values.

We also consider it to be unfair discrimination that the international standards prescribe a different HMF limit for honeys with a low enzyme content (15 mg/kg instead of 40). We are convinced that a limit of 40 is too high for any honey, to guarantee its freshness (Vorwohl, 1969, 1980; Fini and Sabatini, 1972; Piazza and Accorti, 1982; Persano Oddo *et al*, 1985; Accorti *et al*, 1986), but if this value is accepted it should be used for all honeys equally.

**Résumé — Activité diastasique de quelques miels monofloraux.** L'activité diastasique a été déterminée pour 12 groupes de miels monofloraux italiens : *Castanea* (châtaigner), *Robinia* (acacia), *Hedysarum*, *Citrus*, *Eucalyptus*, *Arbutus* (arbousier), *Erica* (bruyère), *Helianthus* (tournesol), *Rhododendron*, *Taraxacum* (pissenlit), *Thymus* (thym) et miellat. La grande variabilité de ce paramètre et le fait qu'il dépend fortement de l'origine botanique du miel ont été confirmés et quantifiés (fig 1, tableau I). En dehors des miels de *Robinia* et de *Citrus*, bien connus pour leur faible indice diastasique, de très faibles valeurs ont été trouvées pour les miels de *Taraxacum*, d'*Erica* et particulièrement d'*Arbutus*,

chez lesquels la diastase peut être absente ou en tout cas inférieure au minimum de 3 prévu par les normes. Les miels d'*Hedysarum*, de miellat, d'*Eucalyptus*, de *Castanea* et encore plus de *Thymus* ont un indice diastasique élevé.

Cette grande variabilité nous conduit à reconsidérer ce paramètre comme indicateur de la fraîcheur du miel, car les limites légales sont trop larges pour certains types de miels et trop restrictives pour d'autres. Finalement, la relation entre l'absorbance à la première lecture (à 5 min) et l'indice diastasique a été étudiée et quantifiée (fig 3, tableau II). Elle est décrite de façon satisfaisante par l'équation linéaire :

$$X_2 = -70,1804 - 95,4185 X_1$$

( $X_2$  = nombre diastasique ;  $X_1$  = absorbance à 5 min).

Cela peut contribuer dans la pratique à simplifier cette analyse.

#### **miel / activité enzymatique / amylase / absorbance**

**Zusammenfassung — Diastase-Aktivität in einigen Einblüten-Honigen.** Für 12 Gruppen italienischer Einblüten-Honige wurde die Diastase-Aktivität bestimmt (*Castanea*, *Robinia*, *Hedysarum*, *Citrus*, *Eucalyptus*, *Arbutus*, *Erica*, *Helianthus*, *Rhododendron*, *Taraxacum*, *Thymus* und Honigtau).

Die große Variabilität dieser Eigenschaft und die große Abhängigkeit von der botanischen Herkunft dieser Honige wurde bestätigt und im einzelnen aufgedgliedert (Abb 1, Tabelle I).

Außer für *Robinia*- und *Citrus*-Honige, deren niedrige Diastase-Werte seit langem bekannt sind, wurden auch für *Taraxacum* (Löwenzahn)-, *Erica*- und besonders für *Arbutus* (Erdbeerbaum)-Honige niedrige

Werte gefunden; die Diastase kann bei diesen sogar fehlen oder niedriger als 3 sein, dem im Standard vorgeschriebenen Mindestwert. *Hedysarum*-, Honigtau, *Eucalyptus*, *Castanea*- und vor allem *Thymus*-Honige haben hohe Diastasewerte.

Diese große Variabilität zwingt uns, die Eignung dieser Eigenschaft als Indikator für die Frische des Honigs neu zu überdenken; denn die gesetzlichen Grenzen sind für einige Honigtypen zu weit, für andere wieder zu eng.

Schließlich wurde die Beziehung zwischen der ersten Ablesung (bei 5 Minuten) und dem Diastase-Index untersucht und quantifiziert (Abb 3 und Tabelle II). Sie kann zufriedenstellend beschrieben werden mit der linearen Gleichung

$$X_2 = -70,1804 - 95,4185 X_1$$

( $X_2$  = Diastasezahl;  $X_1$  = Absorption bei 5').

Das kann zur praktischen Vereinfachung der Analyse beitragen.

## Honig / Enzymaktivität / Amylase / Absorption

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